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Variation in vegetation and microbial linkages with slope aspect in a montane temperate hardwood forest

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Abstract. Plant ecologists have long been interested in aspect-related contrasts of montane forests. Few studies have assessed correlation (linkage) among vegetation strata; fewer have included soil microbial communities. This study assessed contrasts in overstory, spring herbaceous, and soil microbial communities between northeast (NE) - and southwest (SW) -facing slopes in a second-growth West Virginia hardwood forest. We addressed three questions: (1) how do soil microbial, herbaceous layer, and overstory communities vary with slope aspect? (2) do forest vegetation strata and soil microbial communities exhibit linkage? (3) do biotic relationships and linkage vary with slope aspect? Moisture, organic matter, pH, soil NO_3^-, and net nitrification were significantly higher in NE soils; soil NH_4^+ was significantly higher in SW soils, and net N mineralization was virtually identical between aspects. Vegetation communities markedly differed between the slopes. Overall, 29 tree and 118 herbaceous species were encountered, with overstory dominated by sugar maple and sweet buckeye on NE slopes and white oak on SW slopes. The herb layer of the NE aspect was characterized by forbs with fewer graminoids, whereas higher diversity and importance of graminoids were typical for SW slopes. Important microbial groups for NE soils were Gram-positive and Gram-negative bacteria; SW soils were dominated by fungal groups and Gram-negative bacteria associated with environmental stress. Linkage was detected between soil microbes and overstory on the NE slope and between soil microbes and herb layer on the SW slope. We suggest that higher net radiation to the SW slope has created more weathered soil conditions, selecting for microbial groups both adapted to, and maintaining, low N availability (bottom-up control). This has resulted in dominance of NO_3^- in less weathered NE soils and NH_4^+ in more weathered SW soils. Other differences arose from contrasts in humus formation, driven by overstory species composition and maintained by soil moisture (top-down control). Higher moisture of NE soils is more conducive to humus formation, supporting different microbial communities than the SW slopes with drier and often exposed mineral soil. Thus, plants and microbes can form biotic linkages in forest ecosystems which can vary among vegetation strata and with slope aspect.

Key words: forest strata linkage; hardwood forests; slope aspect; soil microbial communities; soil weathering.

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Introduction

Plant ecologists have long been fascinated by the striking contrasts of plant community structure and composition—especially that of forest communities—with slope aspect. Principally focusing on north- versus south-facing comparisons in temperate regions of the northern hemisphere, some of these classic studies have emphasized quantifying aspect-related differences in forest vegetation and on determining aspect-related variation in facets of the physical environment that represent potential mechanisms to explain vegetation patterns (Turesson 1914, Cottle 1932). The seminal studies of forest vegetation by Whittaker in the Smoky Mountains of North Carolina (1956), the Siskiyou Mountains of Oregon and California (1960), and Santa Catalina Mountains of Arizona (Whittaker and Niering 1968) identified slope aspect as a major determiner of environmental gradients relevant to understanding vegetation dynamics. Among earlier work in Europe, Deyl (1940) performed an extensive study of the vegetation of Pop Ivan, a mountain in the Ukrainian Carpathians. He classified vegetation to communities according to several criteria, including slope aspect, referring to south-facing slopes as *Meridionalia* and north-facing slopes as *Septentrionalia*. He also classified soil microorganisms by "physiological groups," and included distinguishing soil types and microclimate as related to plant communities.

The stark aspect-related contrasts in the abiotic environment and associated differences in vegetation are driven primarily by variation in net solar radiation (R_n), which can be ~50% higher on south-facing than on north-facing slopes in the northern hemisphere (Rosenberg et al. 1983, Geiger et al. 2003). This arises largely as function of angle of incidence of R_n, such that south-facing aspects are oriented more directly with the sun and receive both a greater duration and higher intensity of R_n (Tajchman and Lacey 1986, Tajchman et al. 1988, Geiger et al. 2003). Connected to aspect-related variation in R_n are differences in temperature and moisture that result in relatively warmer and drier microclimates on south- versus north-facing slopes (Warren 2008, Beaudette and O'Geen 2009). Less often cited, yet no less important, such variation typically promotes differential rates of soil weathering, with higher R_n resulting in higher weathering rates and, thus, more weathered soil on south-facing than on north-facing slopes (Jenny 1980, Rech et al. 2001).

In one of the more encyclopedic of the earlier studies in North America, Cantlon (1953) examined the vegetation and microclimate of north- and south-facing ridges in central New Jersey. Understandably, he found sharp contrasts in solar radiation and moisture, being higher and lower, respectively, on south-facing slopes. However, although he found profound differences in forest structure and composition related to aspect, he concluded that few species were exclusive to either slope, with differences being primarily related to relative importance. Furthermore, by studying each forest stratum, he found that, whereas there were differences in the tree layer, most variation with slope aspect occurred in the herbaceous layer (Cantlon 1953). In addition to these commonly-reported differences in plant species, aspect-related contrasts in microclimates also have profound effects on the ecophysiology of plants of the herbaceous layer (Neufeld and Young 2014).

Forest strata, particularly the overstory and herb layer, have been shown to vary spatially in ways that suggest a causal connection among them. When this occurs, the strata are said to exhibit *linkage*. This has commonly been reported for overstory and herbaceous strata for a variety of forest ecosystems (Gilliam 2007, McEwan and Muller 2011, Vockenhuber et al. 2011, Jackson et al. 2012, Gilliam and Roberts 2014), with far fewer studies including soil microbial communities in their analyses. Linkage is commonly assessed via multivariate statistics (e.g., ordination), wherein axes scores from separate overstory and herb layer analyses are subjected to correlation and linkage being assumed from significant correlation between axis scores (Barbier et al. 2008). It is possible to employ similar multivariate analytical techniques as those used to assess overstory/herb layer relationships, and applying them toward testing for linkage of forest vegetation with soil microbial communities. This is especially important with regards to soil N processing, which not only affects plant communities but also involves a complex set of pathways involving soil microbes.

Indeed, soil N transformations are largely

governed by microbial activity (Fraterrigo et al. 2006), with one of the more important of these—nitrification—being essentially a solely bacterial process. Consequently, a characterization of the microbial community in response to environmental factors is critical to understanding the underlying mechanisms of vegetation patterns, especially those associated with slope aspect. Microbial processing of N can be viewed as a proximate control on soil N dynamics, with physical/chemical (state) factors, such as parent material and soil weathering, representing ultimate control within which proximate control processes operate. Gilliam et al. (2011) found that gradients of net nitrification in a second-growth, central Appalachian hardwood forest of West Virginia corresponded significantly to a gradient in soil weathering, as evidenced by higher acidity and clay fraction, with less weathered soil having high rates of net nitrification, more weathered soil having barely detectable net nitrification, and intermediately-weathered soils in between these two extremes.

As the organisms primarily responsible for carrying out decomposition of organic matter—releasing CO_2 and plant-available inorganic nutrients—soil microbes represent the foundation of ecosystem structure and function by mediating both energy flow and nutrient cycling (Zak et al. 2003). Soil microbes have been considered as drivers of plant diversity and productivity in terrestrial ecosystems (van der Heijden et al. 2008). Although much of the work on plant/microbe interactions has been carried out in herb-dominated ecosystems (e.g., McCulley and Burke 2004, Kaye et al. 2005, Fitzsimons and Miller 2010, Marshall et al. 2011), increasingly such studies are being carried out also in forests (Eisenlord and Zak 2010). Eisenhauer et al. (2011) found significant positive relationships between soil biota and herb layer diversity of a temperate hardwood forest in Canada; however, they did so by assessing only microbial biomass and basal metabolism, neither examining microbial community composition nor employing multivariate ordination techniques.

The purpose of this study was to assess quantitative differences in plant (overstory and spring herbaceous) and soil microbial communities between northeast- and southwest-facing slopes in a second-growth hardwood forest. More specifically, we addressed the following questions: (1) how do soil microbial, herbaceous layer, and overstory communities vary with slope aspect? (2) do forest vegetation strata and soil microbial communities exhibit linkage? (3) do biotic relationships and linkage vary with slope aspect?

Methods

Study site

The study was carried out at the Beech Fork Lake State Wildlife Area, located in Wayne County, West Virginia (38°18′ N, 82°25′ W). Sampling was carried out on a ridge with slopes of roughly north/northeast (NE) and south/southwest (SW) aspects; elevation ranges of the areas sampled were approximately 178 m to 237 m above mean sea level. Among the nearly 30 tree species encountered via plot sampling, the more common were sugar maple (*Acer saccharum*), sweet buckeye (*Aesculus octandra*), American beech (*Fagus grandifolia*), and white (*Quercus alba*). Of the approximately 150 herb layer species found (including juveniles of woody species), the more abundant dicots were chickweed (*Stellaria media*), Virginia spring beauty (*Claytonia virginica*), harbinger of spring (*Erigenia bulbosa*), cleavers (*Galium aparine*), jumpseed (*Tovara virginiana*), stonecrop (*Sedum ternatum*), Christmas fern (*Polystichum acrostichoides*), skullcap (*Scutellaria nervosa*), and several species of Asteraceae. The most common graminoids were bearded shorthusk (*Brachyelytrum erectum*), heath woodrush (*Luzula multiflora*), and grove bluegrass (*Poa alsodes*). There were also 11 species of sedge (*Carex*) (the more frequent including *C. digitalis*, *C. jamesii* and *C. rosea*). Nomenclature is based on Strausbaugh and Core (1978).

Soils of the study site are primarily of the Gilpin-Upshur complex, which are moderately deep and well-drained, having been formed in acidic material weathered from interbedded shale, siltstone, and sandstone. Gilpin soils comprise fine-loamy, mixed, mesic Typic Hapludults; Upshur soils are primarily fine, mixed, mesic Typic Hapludalfs. These soils occur on ridgetops, benches, and side slopes throughout the region. Soil textures vary from silt loams in the A horizon to channery silty clay loams in the B horizon, and are moderately to slightly acidic,

Table 1. T-test comparisons of soil parameters in northeast (NE)- versus southwest (SW)-aspect soils of Beech Fork Lake State Wildlife Area, WV, including organic matter (OM), water-extractable pH (pH_w), KCl-extractable pH (pH_s), extractable NH_4^+ and NO_3^- (NH_4^+ and NO_3^-, respectively), and net N mineralization and nitrification (net N min and net nit, respectively).

Aspect	Moisture (%)	OM (%)	pHw	pHs	NH_4^+ (μg N/g soil)	NO_3^- (μg N/g soil)	Net N min (μg N/g soil/d)	Net nit (μg N/g soil/d)
NE	37.0 ± 2.0	12.5 ± 1.0	6.69 ± 0.21	5.35 ± 0.22	2.3 ± 0.2	2.1 ± 0.2	0.7 ± 0.4	1.1 ± 0.3
	*	**	**	*	NS	**	NS	**
SW	28.9 ± 2.1	8.3 ± 0.9	5.71 ± 0.23	4.44 ± 0.22	2.6 ± 0.2	0.2 ± 0.1	0.8 ± 0.8	0.2 ± 0.1

Note: NS indicates no significant difference between aspects at $P > 0.05$; * indicates significant difference between aspects at $P < 0.05$; ** indicates significant difference between aspects at $P < 0.01$.

with water-extractable soil pH generally ranging from 5.5 to 6.8 (Table 1).

Field sampling

Sixteen circular 400-m^2 sample plots were located in a regular pattern extending from the NE- to SW-facing aspect of the ridge, yielding eight plots per aspect; sampling took place in April/May 2012. All live trees ≥2.5 cm diameter at breast height (dbh) within each plot were tallied, identified to species, and measured for dbh to the nearest 0.1 cm. Woody individuals taller than 1.0 m and thinner than 2.5 cm dbh were relatively rare and were not included in sampling. Overall, ~800 stems were sampled in the study. The herbaceous layer was assessed by identifying all species of vascular plants ≤1.0 m in height within each entire plot. Abundance of each species was visually estimated using an ordinal scale with classes 1 to 4. Class 1 was for 1–10 individuals/plot, 2 for 11–100, 3 for 101–1000, and 4 for >100 individuals/plot. The herbaceous layer included juveniles of all 29 tree species. In order to capture the main phases of rapidly changing spring species assemblages, the herbaceous layer field sampling was undertaken twice: the first census was from 23 March to 11 April 2012 and included mostly cryptophytes and therophytes; the second census was from 19 to 27 April and was dominated by many species transient into summer vegetation. Species lists of the two sampling campaigns were merged. If a species was encountered in both censuses, higher of the two abundance values was taken for statistical analyses (e.g., 1 and 2 resulted in 2).

Soil was sampled from plot centers in March 2012. After removing humus layers, soil was taken to a 5-cm depth via hand trowel and placed in two 500-mL sterile polyethylene Whirl-Pac bags. The first of these bags was used for determination of soil microbial community structure via phospholipid fatty acid (PLFA) analysis. The second bag was brought back immediately to the Marshall University Weeds and Dirt Laboratory for a variety of initial analyses (see below), including extraction and analysis for NH_4^+ and NO_3^-, followed by controlled incubation for 7 d at 20°C.

Laboratory analyses

Extraction and analysis for NH_4^+ and NO_3^- followed methods described in Gilliam et al. (2011). For both pre-incubation and post-incubation samples, moist soil was extracted with 1N KCl at an extract:soil ratio of 10:1 (v:w). Extracts were analyzed colorimetrically for NH_4^+ and NO_3^- with an AutoAnalyzer 3 automatic analysis system. Net N mineralization rates (in μg N g^{-1} soil d^{-1}) were calculated as post-incubation extractable NH_4^+ and NO_3^- concentrations minus pre-incubation extractable NH_4^+ and NO_3^- concentrations; net nitrification rates (μg N g^{-1} soil d^{-1}) were calculated as post-incubation NO_3^- minus pre-incubation NO_3^-.

Soil moisture was determined gravimetrically by comparing moist soil mass versus oven-dried soil mass (overnight at 70°C). Soil pH was determined on 1:1 weight:volume slurries of both deionized H_2O (pH_w) and 1N KCl (pH_s) using an Orion 3 Star pH meter with a Ross Ultra combination pH electrode. Soil OM was measured from oven-dried soil using the loss-on-ignition method (mass lost over 5 h at 500°C) with a Fisher Isotemp Muffle Furnace.

Composition and biomass of the microbial community were assessed using PLFA analysis, a technique that allows quantification of the viable microbial taxonomic group composition and

biomass at the time of sampling, based on total extractable phospholipids and chemical composition of the constituent fatty acids (Zelles 1999, Kaur et al. 2005, Baker et al. 2009, Frostegård et al. 2011). Methodology for PLFA analysis followed that of Gilliam et al. (2011) with variations to match the specific requirements of the field design. Each sample was homogenized following thawing for 15–30 min. Approximately 5 g moist soil was extracted in a single phase, phosphate-buffered $CHCl_3$–CH_3OH solution to remove PLFAs (Bligh and Dyer 1959). Lipids were separated further by silicic acid chromatography, with phospholipids derivitized in an alkaline solution to form fatty acid methyl esters (FAMEs) (White et al. 1979). The FAMEs were purified further with C18 reverse plasma chromatography, followed by separation and quantification by capillary gas chromatography with a flame ionization detector (Shimadzu 2014GC, Shimadzu, Japan) equipped with a Restek Rtx-1 column (Restek, Bellefonte, PA). Each FAME peak was identified and concentration calculated using a Supelco 37-component FAME mix (Sigma-Aldrich, St. Louis, MO) as standard every three samples. FAMEs present in sample but not in the Supelco mix were identified based on retention times, known standards, and comparison with soil FAME chromatographs generated from independent laboratories using the Microbial Identification Inc. (MIDI) identification system (MIDI, Newark, DE). FAMEs were described using standard nomenclature (IUPAC-IUB 1977), (*A*:*B*n*C*), where *A* is the total number of C atoms, *B* is the number of unsaturated bonds, and *C* is the number of C atoms between the aliphatic end of the molecule and the first unsaturated bond. Prefixes "i" and "a" indicate differential branching, "cy" indicates presence of a cyclopropyl group, and "10me" indicates occurrence of a methyl group at the 10th carbon from the carboxyl end of the molecule. Microbial biomass was calculated as total extractable PLFA (μmol kg^{-1} soil). Fungal/bacterial ratios were calculated (based on μmol kg^{-1} soil) as: (18:2n6 + 18:1n9)/(i14:0 + i15:0 + a15:0 + i16:0 + i17:0 + a17:0 + 16:1n9 + 16:1n7 + cy17:0 + 18:1n7 + 18:1n5 + cy19:0 + 14:0 + 15:0 + 17:0 + 18:0 + 10Me16 + 10Me17 + 10Me18 + i17:1n7). Because 16:1n5 is occasionally reported as a marker for bacteria, it was not included in this calculation (Tunlid and White 1992).

Data analyses

Data for overstory were summarized into importance value (IV, %) tables for each aspect separately, with IVs calculated as the mean of relative basal area and relative density for each woody species. Data for the herb layer were similarly summarized into IV tables, except using relative cover class. Soil microbial communities were compared between aspects as mean mole fraction (%) of each of the 35 more abundant (out of total of 75 identified) FAMEs via t-tests; means of total bacterial and fungal biomass, ratio of fungal to bacterial biomass, and ratio of the FAMEs cy19 to 18:1n7c—sometimes used as an index of environmental stress for microbes (Kaur et al. 2005, but see also Frostegård et al. 2011 for a critique)—were also compared between aspects with t-tests.

Means of variables determined by initial soil analyses (i.e., soil moisture, pH_w, pH_s, OM, extractable NH_4^+/NO_3^-) and vegetation variables (i.e., tree basal area, tree density, tree and herb richness) were compared between aspects via t-tests. Following one-week laboratory incubations, daily means of net N mineralization and nitrification were also compared between aspects via t-tests (Zar 2009).

To examine species and environmental contrasts between slope aspects, quantitative data for all groups—overstory, herb layer, and soil microbes—were analyzed separately along with a variety of soil variables, including moisture, organic matter, and inorganic and mineralizable N, using canonical correspondence analysis (CCA) (Canoco for Windows 4.5). To assess linkage among communities, and its potential variation with slope aspect, CCA was also performed on community/soil data for each slope aspect separately. Linkage was determined by testing for correlation among CCA axis scores for overstory, herb layer, and microbial communities using Pearson product-moment correlation (Zar 2009).

Results

Of the 29 woody species found in all 16 0.04-ha sample plots in the study, 15 occurred on the NE-facing aspect, with 24 occurring on the SW-facing

Table 2. T-test comparisons of plant parameters in northeast versus southwest aspects of Beech Fork Lake State Wildlife Area, WV, including overstory basal area and density (BA and D, respectively), and species richness for overstory and herbaceous layer (S_{TREE} and S_{HERB}, respectively).

Aspect	BA (m^2/ha)	D (stems/ha)	S_{TREE} (species/plot)	S_{HERB} (species/plot)
Northeast	28.3 ± 2.5	413 ± 39	5 ± 1	48 ± 2
	NS	**	**	**
Southwest	21.6 ± 2.4	825 ± 53	9 ± 1	56 ± 2

Note: NS indicates no significant difference between aspects at $P > 0.05$; * indicates significant difference between aspects at $P < 0.05$; ** indicates significant difference between aspects at $P < 0.01$.

aspect, and there were significant differences between aspects in overstory species density (mean plot richness), which was 9 and 5 tree species per plot for SW and NE aspects, respectively (Table 2). The overstory of the NE-facing aspect was dominated primarily by two species, *Acer saccharum* and *Aesculus octandra*, which, in combination, represented nearly 70% of importance value (Table 3). By contrast, the SW-facing aspect was more evenly co-dominated by *Quercus alba*, *Q. rubra*, *Fagus grandifolia*, and *Acer saccharum* (Table 4). Another common species on the SW slope was *Acer rubrum*, which was not recorded on the NE slope. Dominant species contrasted sharply between aspects: *Aesculus octandra*, a co-dominant on the NE-facing aspect, was rare on the SW-facing aspect; *Q. alba*, the dominant species on the SW-facing aspect, was of minor importance on the NE-facing aspect. In addition, whereas tree basal area did not vary significantly between slope aspects, tree density was nearly 80% higher on the SW slope than the NE slope (Table 2).

Of the 147 herbaceous layer species (including juveniles of woody species) found in sample plots of the study, 86 were found on the NE-facing aspect, whereas 113 were found on the SW-facing aspect, resulting in significantly higher species richness of the herb layer on SW than NE slopes (56 versus 48 species per plot, respectively; Table 2). Whereas the NE aspect can be characterized by three highly abundant species *Stellaria media*, *Erigenia bulbosa*, and *Claytonia virginica*, herbaceous vegetation of the SW aspect was more evenly composed of various graminoid and forb species, e.g., *Luzula multiflora*, *Scutellaria nervosa*, *Carex digitalis* and *Poa cuspidata* (Table 5).

Although total bacterial biomass did not vary with aspect, fungal biomass was significantly higher on SW slopes than on NE slopes, which resulted in a significantly higher fungal:bacterial (F:B) biomass ratio (Table 6). The more predominant microbial fatty acid methyl ester types (FAMEs) of all soils at this site, based on % mole fraction, were 18:1n7c, 16:0, and 16:1n7c, none of

Table 3. Importance values (IV) of overstory species of the NE-facing aspect of a second-growth hardwood stand, Beech Fork Lake State Wildlife Area, West Virginia. IV of each species was calculated as the mean of relative basal area (BA) and density (D).

Species	Basal area (m^2/ha)	Density (stems/ha)	Relative BA (%)	Relative D (%)	IV (%)
Acer saccharum	10.4	191	36.7	46.2	41.5
Aesculus octandra	7.6	100	27.0	24.2	25.6
Fagus grandifolia	3.0	22	10.4	5.3	7.9
Fraxinus americana	2.0	19	7.2	4.5	5.9
Carya ovata	1.2	13	4.4	3.0	3.7
Platanus occidentalis	1.1	13	3.9	3.0	3.5
Lindera benzoin	0.0	19	0.0	4.5	2.3
Prunus serotina	1.0	3	3.7	0.8	2.2
Acer negundo	0.3	13	1.1	3.0	2.0
Liriodendron tulipifera	0.6	3	2.0	0.8	1.4
Carya glabra	0.4	3	1.6	0.8	1.2
Tilia americana	0.4	3	1.4	0.8	1.1
Other	0.2	12	0.6	3.1	1.9
Total	28.3	413	100	100	100

Table 4. Importance values (IV) of overstory species of the SW-facing aspect of a second-growth hardwood stand, Beech Fork Lake State Wildlife Area, West Virginia. IV of each species was calculated as the mean of relative basal area (BA) and density (D).

Species	Basal area (m^2/ha)	Density (stems/ha)	Relative BA (%)	Relative D (%)	IV (%)
Quercus alba	10.7	81	49.6	9.9	29.8
Fagus grandifolia	2.0	197	9.2	24.0	16.6
Acer saccharum	1.7	172	7.8	21.0	14.4
Q. rubra	3.9	38	18.2	4.6	11.4
A. rubrum	0.3	113	1.2	13.7	7.5
Cornus florida	0.2	75	0.9	9.2	5.0
Pinus rigida	1.8	9	8.3	1.1	4.7
Carya glabra	0.3	22	1.5	2.7	2.1
Carpinus caroliniana	0.1	28	0.4	3.4	1.9
Fraxinus americana	0.2	16	0.7	1.9	1.3
Other	0.3	66	2.2	8.6	5.2
Total	21.6	816	100.0	100.0	100.0

which varied with slope aspect (Table 7). There were sharp contrasts, however, between slope aspects in several other FAMEs, with 19 out of the top 35 FAMEs being significantly different between NE- versus SW-slope aspects (Table 7). Those significantly higher on NE slopes included 10Me16, a15, cy17, i17:1n7 and a17. Those significantly higher on the SW slope included 18:1n5, cy19, 18:2n6c, and 18:1n9c (Table 7).

Several soil variables varied significantly with

Table 5. Importance values (IV) of the top 30 (based on IV) herb layer species of NE- and SW-facing aspects of a second-growth hardwood stand, Beech Fork Lake State Wildlife Area, West Virginia. IV of each species is calculated as relative cover. Herb layer species are designated as forbs (F), graminoids (G), and juveniles of woody species (juv.).

NE-facing aspect		SW-facing aspect	
Species	IV	Species	IV
Stellaria media (F)	4.1	*Luzula multiflora* (G)	2.9
Erigenia bulbosa (F)	4.0	*Scutellaria nervosa* (F)	2.8
Claytonia virginica (F)	3.4	*Carex digitalis* (G)	2.6
Acer saccharum (juv.)	2.8	*Poa cuspidata* (G)	2.6
Dentaria concatenata (F)	2.8	*Brachyelytrum erectum* (G)	2.2
Hydrophyllum macrophyllum (F)	2.6	*Carex wildenowii* (G)	2.2
Tovara virginiana (F)	2.6	*Fraxinus americana* (juv.)	2.2
Carex jamesii (G)	2.4	*Potentilla canadensis* (F)	2.2
Polystichum acrostichoides (F)	2.4	*Houstonia caerulea* (F)	2.0
Poa alsodes (G)	2.4	*Parthenocissus quinquefolia* (F)	2.0
Anemonella thalictroides (F)	2.1	*Sedum ternatum* (F)	2.0
Carex hirsutella (G)	2.1	*Viola hirsutula* (F)	2.0
Ulmus rubra (juv.)	2.1	*Carex blanda* (G)	1.9
Carex laxiflora (G)	2.0	*Krigia biflora* (F)	1.9
Caulophyllum thalictroides (F)	2.0	*Oxalis violacea* (F)	1.9
Galium aparine (F)	2.0	*Disporum lanuginosum* (F)	1.8
Ranunculus recurvatus (F)	1.8	*Polystichum acrostichoides* (F)	1.8
Adiantum pedatum (F)	1.7	*Acer rubrum* (juv.)	1.6
Asarum canadense (F)	1.7	*Carex rosea* (G)	1.6
Lindera benzoin (juv.)	1.7	*Cunila origanoides* (F)	1.6
Phlox divaricata (F)	1.7	*Podophyllum peltatum* (F)	1.6
Ranunculus micranthus (F)	1.7	*Prunus* sp. (juv.)	1.6
Viola pubescens (F)	1.7	*Uvularia sessilifolia* (F)	1.6
Circaea lutetiana ssp. *canadensis* (F)	1.5	*Cinna arundinacea* (G)	1.5
Galium triflorum (F)	1.5	*Amelanchier arborea* (juv.)	1.3
Parthenocissus quinquefolia (F)	1.5	*Carex albursina* (G)	1.3
Sedum ternatum (F)	1.5	*Toxicodendron radicans* (F)	1.3
Actaea pachypoda (F)	1.4	*Vaccinium pallidum* (F)	1.3
Carex albursina (G)	1.4	*Fagus grandifolia* (juv.)	1.2
Fraxinus americana (juv.)	1.4	*Galium aparine* (F)	1.2

Table 6. T-test comparisons of microbial parameters in northeast- versus southwest-aspect soils of Beech Fork Lake State Wildlife Area, WV, including total bacterial and fungal biomass, fungi/bacteria (F/B) ratio, and FAME ratio of cy19/18:1n7c, used as an indicator of microbial environmental stress.

Aspect	Bacteria (nmol/g dry soil)	Fungi (nmol/g dry soil)	F/B ratio	cy19/18:1n7c ratio
Northeast	76.1 ± 9.4	14.9 ± 1.7	0.20 ± 0.01	0.51 ± 0.04
	NS	**	*	*
Southwest	89.9 ± 12.7	24.6 ± 3.8	0.27 ± 0.01	0.88 ± 0.15

Note: NS indicates no significant difference between aspects at $P > 0.05$; * indicates significant difference between aspects at $P < 0.05$; ** indicates significant difference between aspects at $P < 0.01$.

slope aspect. Moisture, organic matter, pH (both H_2O- and KCl-extractable) were all significantly higher in NE-aspect soils (Table 1). Soil N contrasted sharply between NE and SW aspects, including the dominant form of available N. Whereas total inorganic (extractable) N was ~93% NH_4^+ in SW soils, inorganic N was essentially equally distributed between NH_4^+ and NO_3^- in NE soils; extractable NH_4^+ did not vary between aspects. These contrasts were reflected also in potential net N mineralization and nitrification rates; net N mineralization was virtually identical between aspects, whereas net nitrification exceeded N mineralization in NE soils and was nearly undetectable in SW soils (Table 1).

Table 7. Mean mole fraction percentage (relative abundance) of 35 fatty acid methyl ester (FAME) groups at Beech Fork Lake State Wildlife Area, WV. Significant differences between slope aspects indicated by bold type for higher mean and asterisks as the result of t-tests: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; no asterisk indicates no significant difference between aspects.

FAME	NE aspect, % mole	Significance	SW aspect, % mole
18:1n7c	11.75		11.07
16:0	10.30		11.17
16:1n7c	**6.17**	*	5.55
10Me16	**6.14**	***	3.56
18:1n9c	6.05	***	**9.08**
cy19	5.98	**	**8.83**
i15	5.72		5.64
16:1n5c	4.95		4.39
a15	**4.87**	*	3.65
cy17	**3.10**	*	2.54
i17:1n7	**2.97**	****	2.22
a17	**2.55**	**	2.20
18:0	2.28		2.51
10Me15	2.25		2.23
10Me18	2.18		2.53
i17	**1.83**	****	1.43
16:1n9c	**1.65**	*	1.45
18:2n6c	1.24	*	**2.46**
18:1n5c	1.20	*	**1.40**
11Me18:1n7c	**0.91**	****	0.48
i14	**0.90**	*	0.43
a15:1	0.72		0.68
10Me17	0.63	**	**0.91**
b19:1a	0.63	**	**1.02**
17:1n9c	**0.62**	***	0.21
14:0	0.58		0.62
i16:1a	**0.58**	**	0.44
17:1a	0.55		0.55
20:4n6	0.54		0.64
22:0	0.52		0.61
18:3n3	0.52		0.31
12Me16	0.49		0.75
21/22:6n3	**0.48**	**	0.24
15:1n5c	0.45		0.37
9Me16	**0.45**	****	0.24

Canonical correspondence analysis (CCA) revealed patterns of both similarity and contrast regarding species variation and potential responses of plant and microbial communities to environmental factors. Sample plots separated widely in ordination space with respect to slope aspect for overstory and herb layer communities, with greater variability among SW plots than among NE plots for both forest vegetation strata (Figs. 1 and 2). Based on vector length, environmental variables important in explaining species variation were also similar among vegetation strata, with soil organic matter, moisture, and extractable NO_3^- pools being more important than other factors, confirming reported contrasts (Table 1). Sample plots were generally well-separated for microbial communities, though not as widely as for plant community data; similar to plant community data was the greater variability in soil microbial composition among SW plots than among NE plots (Fig. 3). In contrast to plant communities, however, microbial communities appeared to respond most sensitively to gradients of soil acidity, especially that associated with KCl-extractable pH (pH_s), which averaged 5.35 ± 0.22 and 4.44 ± 0.22, for NE and SW soils, respectively (Table 1).

Plant and microbial community, along with

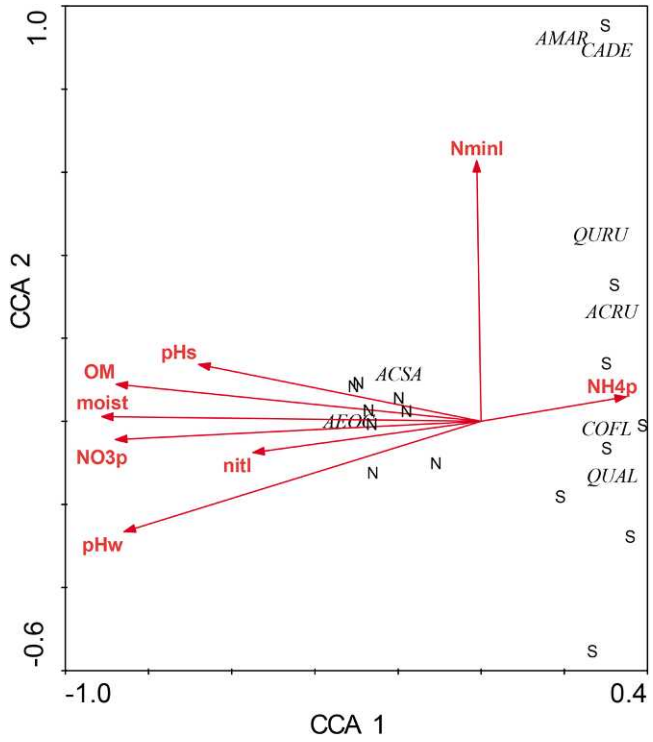

Fig. 1. Canonical correspondence analysis of overstory species at Beech Fork Lake State Wildlife Area, WV. Plot locations in ordination space are indicated by N (northeast slopes) and S (southwest). Environmental vectors are as follows: moisture (moist), organic matter (OM), water-extractable pH (pH_w), KCl-extractable pH (pH_s), extractable NH_4^+ and NO_3^- (NH_4^+ and NO_3^-, respectively), and net N mineralization and nitrification (NminI and nitI, respectively). Overstory species codes are as follows: *Aesculus octandra* (AEOC), *Acer saccharum* (ACSA), *Quercus rubra* (QURU), *Acer rubrum* (ACRU), *Cornus florida* (COFL), *Amelanchier arborea* (AMAR), *Castanea dentata* (CADE), and *Quercus alba* (QUAL).

environmental data, were also subjected to CCA for each slope aspect separately to assess linkage (see *Methods*). Axis scores from separate overstory, herb layer, and microbial CCA analyses were subjected to Pearson product-moment correlation analysis, with significant correlations accepted as evidence of linkage among groups. For the NE aspect, only axis 1 scores from the overstory and microbial communities were significantly correlated (Table 8); for the SW, only axis 1 scores from the herb layer and microbial communities were correlated (Table 9).

Discussion

Aspect-related contrasts in biotic communities and soil characteristics

As expected, there were notable differences in overstory and herb layer communities between NE- and SW-facing aspects of this second-growth hardwood forest of western West Virginia. Consistent with findings of the classic aspect study of Cantlon (1953), differences for overstory and herb layer communities were as much related to relative dominance/importance of species as to absolute differences in species composition. Both the overstory and herb layer commmunities had Sorensen similarity coefficients of >0.50 in comparing total species composition of each stratum between aspects (data not shown). Also as expected, soil microbial composition varied greatly between NE- and SW-facing aspects at this site, consistent with results found by Gilliam et al. (2011), who reported sharp aspect-related contrasts in microbial communities in soils of a watershed of the Fernow

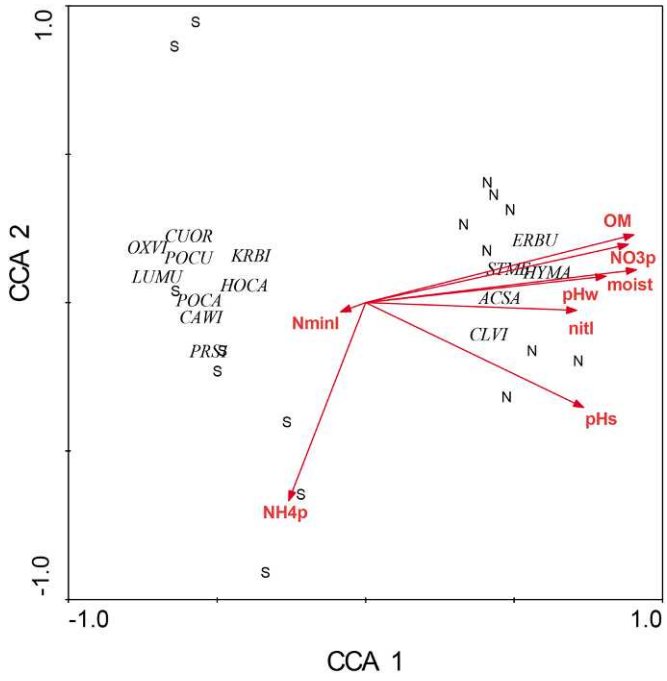

Fig. 2. Canonical correspondence analysis of herbaceous layer species at Beech Fork Lake State Wildlife Area, WV. Plot locations in ordination space are indicated by N (northeast slopes) and S (southwest). See Fig. 1 for explanation of environmental vectors. Herbaceous layer species are as follows: *Cunila origanoides* (CUOR), *Oxalis violacea* (OXVI), *Poa cuspidata* (POCU), *Krigia biflora* (KRBI), *Luzula multiflora* (LUBU), *Houstonia caerulea* (HOCA), *Potentilla canadensis* (POCA), *Carex wildenowii* (CAWI), *Prunus serotina* (PRSE), *Erigenia bulbosa* (ERBU), *Stellaria media* (STME), *Hydrophyllum macrophyllum* (HYMA), *Acer saccharum* (ACSA), and *Claytonia virginica* (CLVI).

Experimental Forest, WV.

Whereas the overstory on the NE slope was dominated by *Acer saccharum* and *Aesculus octandra*, which combined for nearly 80% of IV, the top four species on the SW slope (*Q. alba*, *F. grandifolia*, *A. saccharum*, and *Q. rubra*) combined for just over 70% of IV. A common species on the SW slope was *Acer rubrum*, which was not recorded on the NE slope. *Acer rubrum*, which occured abundantly as young saplings, is relatively short-lived tree producing abundant small seeds, and can be indicative of a younger stand age stage (Fralish and Franklin 2004). Although it was of relatively low basal area on the SW slope, it was the species of third highest density (Table 4). Studies in other eastern hardwood forests have shown a replacement of upland *Quercus* species by *A. rubrum* (Alexander and Arthur 2010). The relatively high density of *Acer saccharum* on both aspects, despite sharply contrasting soil characteristics (Table 1), is consistent with its observed wide distribution across a variety of site types in the eastern deciduous forest (Fralish and Franklin 2004).

Results of CCA for each community separately (Figs. 1–3) confirm these aspect-related contrasts, which were associated with variation in several soil characteristics. Indeed, virtually all soil variables, with the exception of extractable NH_4^+ and net N mineralization rates, varied significantly with slope aspect, and in ways predicable based on aspect-related differences in forest microclimate. This is primarily related to higher R_n on SW slopes which drives higher temperatures, evapotranspiration, and soil weathering, leading to less fertile, more acidic soils (Rech et al. 2001, Geiger et al. 2003, Beaudette and O'Geen 2009). Indeed, soil N contrasted sharply between NE and SW aspects in the dominant form of available N. Total inorganic N was >90% NH_4^+ in SW soils, but was essentially equally distributed between NH_4^+ and NO_3^- in NE soils. Furthermore, net nitrification exceeded N mineralization in NE

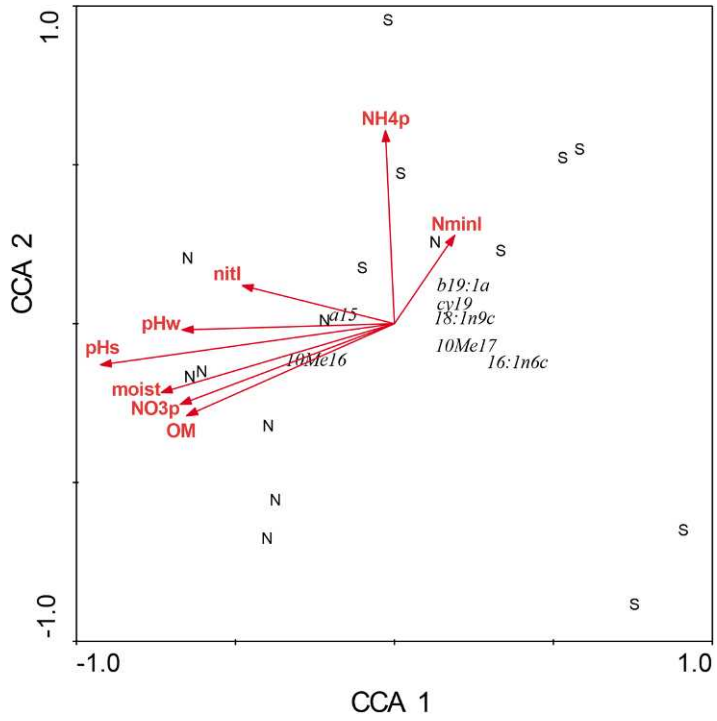

Fig. 3. Canonical correspondence analysis of microbial composition at Beech Fork Lake State Wildlife Area, WV. Plot locations in ordination space are indicated by N (northeast slopes) and S (southwest). See Fig. 1 for explanation of environmental vectors.

soils and was nearly undetectable in SW soils. Thus, similarities in aspect-related contrasts in soil N variables and in microbial community characteristics were consistent with findings of Gilliam et al. (2011) that reported higher F:B ratios, the predominance of fungal PLFA markers, and lower rates of soil N processing to be associated with SW-facing slopes at Fernow Experimental Forest, West Virginia.

Patterns of linkage among biotic communities

As reviewed in Barbier et al. (2008) and Gilliam

Table 8. Correlation matrix of axis scores from canonical correspondence analysis (CCA) of overstory ("Tree"), herbaceous layer ("Herb"), and microbial (PLFA) communities of the northeast aspect of Beech Fork Lake State Wildlife Area, WV. "1″ and "2" indicate CCA axes 1 and 2, respectively, for each community type. The top value in each pair is Pearson product-moment correlation coefficient (r) and the corresponding P value appears below. Significant correlations at $P < 0.05$ are indicated by bold type.

CCA axis	Herb1	Herb2	PLFA1	PLFA2	Tree1
Herb2	0.10				
	0.82				
PLFA1	−0.26	0.61			
	0.53	0.11			
PLFA2	−0.33	−0.47	−0.01		
	0.42	0.25	0.99		
Tree1	−0.10	0.31	**0.73**	−0.08	
	0.82	0.46	**0.05**	0.86	
Tree2	0.01	0.62	0.12	−0.28	0.12
	0.99	0.11	0.77	0.50	0.78

Table 9. Correlation matrix of axis scores from canonical correspondence analysis (CCA) of overstory ("Tree"), herbaceous layer ("Herb"), and microbial (PLFA) communities of the southwest aspect of Beech Fork Lake State Wildlife Area, WV. "1" and "2" indicate CCA axes 1 and 2, respectively, for each community type. The top value in each pair is Pearson product-moment correlation coefficient (r) and the corresponding P value appears below. Significant correlations at $P < 0.05$ are indicated by bold type.

CCA axis	Herb1	Herb2	PLFA1	PLFA2	Tree1
Herb2	0.06				
	0.89				
PLFA1	**0.90**	0.31			
	0.01	0.46			
PLFA2	0.43	−0.59	0.03		
	0.29	0.12	0.95		
Tree1	−0.16	−0.21	0.08	−0.20	
	0.73	0.62	0.85	0.63	
Tree2	0.10	−0.03	0.03	−0.01	−0.03
	0.82	0.95	0.94	0.99	0.95

and Roberts (2014), a common methodology for assessing linkage among forest strata has been to (1) sample strata in the same plots in the field, (2) perform ordination analysis on plot data for each strata separately, and (3) perform correlation analyses on ordination axes scores for individual plots. Because ordination analysis reveals patterns of species variation, significant correlations among ordination axis scores of forest strata provides evidence of linkage between strata. This is especially the case for the first axis, as it typically explains far more variation in species' pattern than other axes (Barbour et al. 1999). In our study, because we also sampled the soil microbial community on these same plots, we can extend this approach using ordination of soil microbial data.

Based on the results of correlation analysis of CCA axis 1 and 2 scores (CCA1 and CCA2, respectively; ordination figures not shown) of each community type, the overstory and herb layer did not exhibit linkage with each other for either NE or SW aspects, i.e., there were no significant correlations for either aspect among vegetation strata for either axis (Tables 8 and 9). Gilliam et al. (1995) suggested a mechanism for linkage among forest vegetation that emphasized the importance of gradients of environmental factors. In essence, linkage is predicted to arise when forest strata respond to similar gradients in a similar fashion, a contention that has been supported in the literature (Barbier et al. 2008, Burton et al. 2011, McEwan and Muller 2011, Vockenhuber et al. 2011, Jackson et al. 2012, Bartels and Chen 2013). The lack of linkage between overstory and herb layer for both slope aspects is also consistent with this prediction, considering that these strata appear to respond to different environmental gradients: the herb layer on the NE aspect was more influenced by extractable NO_3^- and soil OM, whereas the overstory was influenced more by soil pH and moisture. For the SW aspect, the overriding environmental factor for the herb layer was soil pH, but the overstory appeared more influenced by extractable NO_3^- and soil moisture.

In contrast, the microbial community did exhibit linkage with forest vegetation strata, and did so in a way that varied both with strata and slope aspect. For the NE aspect, CCA1 of the microbial community was signficantly correlated with CCA1 of the overstory ($r = 0.73$, $P < 0.05$; Table 8). For the SW aspect, CCA1 of the microbial community was significantly correlated with CCA1 of the herb layer community ($r = 0.90$, $P < 0.01$; Table 9). Again, these results are consistent with the environmental gradient hypothesis of Gilliam et al. (1995), i.e., the microbial community and the overstory were both primiarily influenced by soil pH on the NE aspect, with both also influenced primarily by pH on the SW aspect. Working in a temperate deciduous forest in Germany, Thoms et al. (2010) also found significant relationships between tree species and soil microbial communities that were driven largely by variation in soil pH, consistent with our findings for the NE aspect and with conclusions of an extensive review on the influence of tree species on litter and soil microbial communities by Prescott and Grayston

(2013).

Several studies have demonstrated the reciprocal relationships between plant and soil microbial communities. Indeed, soil microbial diversity has long been shown to be essential in maintaining sustainability of soils (Kennedy and Smith 1995), enhancing and maintaining soil nutrient availability for plant species. On the other hand, plants have been shown to directly influence microbial diversity in soil (see Garbeva et al. 2004 for an extensive review), even at the level of individual plant genotypes (Schweitzer et al. 2008). Westover et al. (1997) showed that plants can affect soil microbial community structure by altering rhizosphere populations of non-symbiotic bacteria and fungi, although this effect has been shown in other studies to be more profound on bacterial than on fungal communities (Buyer et al. 2002). Broughton and Gross (2000) demonstrated that not only did patterns of plant diversity affect soil microbial communities, but did so in a way that varied substantially along a productivity gradient in herb-dominated old fields. Results from the present study confirm that these intricate plant-microbe relationships can occur among both tree and herb layer species, and furthermore that these relationships can vary with slope aspect.

Synthesis and implications for global change

Classic aspect-related studies of forest communities relate variation in species composition to environmental factors driven by sharp contrasts in solar radiation (R_n): higher photon flux density, higher temperatures, and lower moisture (from higher evapotranspiration) associated with S-facing aspects (Cantlon 1953, Geiger et al. 2003, Warren 2008, Beaudette and O'Geen 2009). We suggest another factor, also directly influenced by R_n, as an important driver in creating and maintaining aspect-related differences in forest biota: soil weathering.

Work in other montane hardwood stands of West Virginia (i.e., Fernow Experimental Forest) has highlighted the importance of weathering as a potentially ultimate control on soil N dynamics, one that is primarily related to aspect-related variation in microbial communities (Gilliam et al. 2011). They found considerable evidence to suggest that variation in net N mineralization and nitrification in their study watershed (WS4) was the product of differential rates of mineral weathering during the process of soil formation. Earlier work by Tajchman (Tajchman and Lacey 1986, Tajchman et al. 1988) at WS4 had demonstrated over a 35-yr period (1948–1982) that net radiation—a principle driver in weathering of primary and secondary soil materials (Jenny 1980, Birkeland 1999, Rech et al. 2001)—was highest on southwest-facing upper slopes of the watershed and lowest on the north/northeast facing slopes. Notably, Gilliam et al. (2011) found aspect-related variation in soil microbial communities similar to that found in our study, i.e., significantly greater dominance of bacterial groups (especially those associated with nitrification) on NE-facing slopes and greater dominance of fungal groups on SW-facing slopes.

Weathering of primary and secondary soil materials typically results in release of H^+ and associated acidification of soils (Birkeland 1999) and mobility of Al^{3+} (Gilliam et al. 2005), both of which displace nutrient cations from exchange sites, thus decreasing soil fertility. This not only creates nutrient limitation for plant species, but also stress conditions, especially when combined with higher evapotranspiration (via higher R_n), for microbes. Work by Kaur et al. (2005) has demonstrated that higher FAME ratios of cy19/18:1n7c are strong indicators of microbial stress. On the other hand, Frostegård et al. (2011) offered a compelling critique of the use of this ratio as indicative of microbial stress, so such conclusion should be made with caution. Certainly, the significantly higher ratios for SW versus NE slopes in our study (0.88 and 0.51, respectively), along with significantly higher acidity in SW soils, is consistent with greater stress for microbial communities on SW slopes (Table 6).

Such conditions, including both moisture and nutrient limitations, also create stress for plants, particularly those of the herb layer, which often form mutualistic relationships with fungi, i.e., mycorrhizae, to aid in access to both soil water and essential nutrients, such as N and P. Although mycorrhizal associations are common among vascular plants, they are especially pronounced in plants in stressed environments (Sakamoto et al. 2004, Wang and Qiu 2006). Using the same PLFA analysis used in this study, Nilsson et al. (2005) reported notable increases in

fungal biomass along a natural gradient in soils of Swedish forests, with increases related to decreasing nutrient availability, similar to our findings. Furthermore, they found that the FAME most closely associated with ectomycorrhizae was 18:2n6, one of the FAMEs that were significantly higher in SW soils (Fig. 1). Olsson (1999) also concluded that 18:2n6 is a good predictor of mycelial biomass of ectomycorrhizal fungi, which are not only adapted to low fertility conditions, but can also further maintain them (Gilliam et al. 2005).

We therefore suggest that tree species of the NE aspect represent top-down control on microbial communities, which respond sensitively to inputs of high quality litter from dominant tree species (e.g., *Acer saccharum*, *Aesculus octandra*), leading to the observed linkage between soil microbes and overstory for the NE aspect (Table 8). We further suggest that the more weathered status of soils of the SW aspect represents bottom-up control on microbial communities, with greater acidity and lower fertility that also select for a characteristic herb community, resulting in the linkage between soil microbes and the herb layer for the SW aspect (Table 9).

These interactions represent feedbacks between above- and belowground processes that are important in maintaining structure and function of forests ecosystems. Such feedbacks are also potentially sensitive to global change, especially relative to temporal increases in N deposition and global warming. The N homogeneity hypothesis, as first articulated by Gilliam (2006) and supported in the literature (e.g., Hedwall et al. 2011, García-Palacios et al. 2012; see Gilliam 2014 for recent review), is based on the observation that spatial heterogeneity in soil resources (i.e., N availability) drives, to a large degree, spatial patterns of herb layer species, with lower heterogeneity (greater homogeneity) leading to lower variability in species composition. Data from this study are consistent with this observation. Using coefficient of variation (CV) as an index of heterogeneity, N availability is notably lower on the NE slope: CV for extractable NO_3^- was 30% versus 156% for NE versus SW aspects, respectively; for net N mineralization, this was 144 versus 294, and for net nitrification this was 66 versus 175, respectively. These contrasts are consistent with the lower area occupied by points in ordination space for not only the herb layer (Fig. 2), but also for the overstory (Fig. 1) and microbial groups (Fig. 3).

Because N deposition is projected to increase in annual rate and spatial extent (Bobbink et al. 2010), future scenarios should include consideration of how increased availability of soil N might alter plant—microbial feedbacks. Using an experimental approach, Wei et al. (2013) found compelling evidence that increased N deposition can weaken plant–microbe interactions. Experimental additions of N significantly decreased both bacterial (B) and fungal (F) microbial biomass, but particularly fungal biomass, resulting in significant decreases in F:B ratios with added N. They further concluded that N-mediated acidification, rather than change in litter C/N ratios, was most responsible for weakening these interactions (Wei et al. 2013). Our field-based contrasts between NE and SW aspects in soil N and PLFA data are consistent with this pattern.

These contrasts between aspects also have implications for projected change in microbial function due to climate change, especially in the response of soil temperature and moisture to future global warming scenarios. Whitby and Madritch (2013) used intact cores of Appalachian Mountain forest soil in manipulating temperature to simulate climate warming and its effects on soil C and N metabolism, both of which are primarily a function of microbial activity. Across all soils of the study, even modest experimental warming increased rates of soil respiration, as well as the proportion of available soil C being respired (i.e., amount of respiration as a function of soil C). In addition, experimental warming increased the proportion of soil N that was mineralized (i.e., amount of N mineralized as a function of soil N).

Conclusions

There were sharp differences in plant communities related to slope aspect at this second-growth hardwood forest site, a finding not in itself especially novel considering it corroborates studies dating back to the early 20th century (Turesson 1914, Cottle 1932, Deyl 1940). However, there were equally contrasting aspect-related differences in soil microbial communities, something rarely reported in the literature (Carletti et

al. 2009). We suggest that higher net radiation to the SW slope has created more weathered soil conditions, selecting for microbial groups both adapted to—and maintaining—low nutrient, especially N, availability, which we consider bottom-up control of the soil microbial community. Other differences likely arose from contrasts in humus formation, driven by species composition and maintained by soil moisture, which we consider top-down control of soil microbes. Higher moisture of NE soils is more conductive to humus formation, supporting different microbial communities than the SW slopes with drier and more exposed mineral soil. Thus, plant and microbial communities can form biotic linkages in forest ecosystems, something which can vary among forest strata and with slope aspect. Results from this study has the potential to inform models for climate change regarding plant-soil feed backs, especially with respect to increasing N availability from increased N deposition and changes in soil temperature and moisture from global warming.

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